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Novel Substituted Indazoles Towards Potential Antimicrobial Agents

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ABSTRACT

The *In vitro* antimicrobial properties of a series of N-methyl-3-aryl indazoles (5a-5j) were screened. In this present work, we describe our efforts towards the development of potent antimicrobial activity of synthesized indazole derivatives. The antimicrobial activities of the prepared compounds were investigated against four bacterial strains: *Xanthomonas campestris*, *Escherichia coli*, *Bacillus cereus*, *Bacillus megaterium*, and a fungal strain *Candida albicans*. The biological evaluation studies of these indazole derivatives revealed that some of these tested compounds have shown moderate to good *In vitro* antimicrobial activities.

Keywords: Indazole, Antimicrobial activity, Well diffusion.

INTRODUCTION

Due to the omnipresent nature of nitrogen containing heterocycles they are the key scaffolds of many biologically important molecules and pharmaceutical products. These nitrogen atoms containing heterocycles are part of the world's largest selling drugs.[1] Nowadays, it is well realized that indazole based motifs are an imperative family of nitrogen containing heterocycles with a broad array of agricultural, biological and industrial applications.[2] The structurally varied indazole analogs have been achieved enormous consideration previously, just as in ongoing period, as a result of their wide scope of biological properties, such as anti-hypertensive,[3] anti-inflammatory,[4] anti-HIV,[5] antimicrobial,[6] anti-angiogenesis,[7] anticancer,[8] anti-tubercular[9], neuroprotective[10] and anti-protozoal[11] activities (Fig. 1). Some indazole derivatives were also reported as estrogen[12] and 5-HT1A receptors.[13] Additionally, in the area of modern drug design and discovery, indazoles are useful bioisosteres for benzimidazoles and indoles.[14] Especially, a number of indazole derivatives were reported as potent anti-bacterial and antimicrobial agents (Figure 2).[15-20]

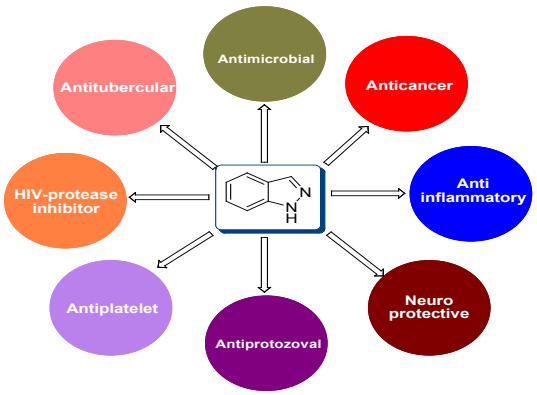


Fig. 1. Pharmacological properties of derivatives with indazole scaffold

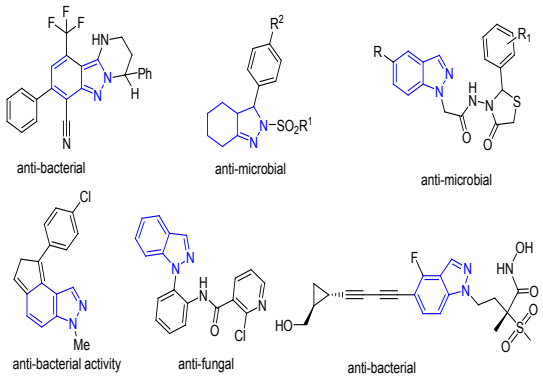
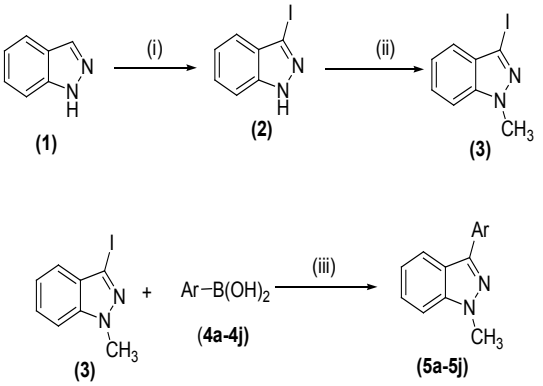


Fig. 2. Some antimicrobial and antibacterial, antifungal agents with indazole skeleton

Moreover, the usage of many antimicrobial agents is limited not only by the quickly rising drug resistance but also by the substandard status of current treatments of fungal and bacterial infections. Thus, the development of new compounds for resistance to bacteria and fungi has become one of the most significant areas of antimicrobial research. The increasing resistance towards the existing antimicrobial agents resulted in an essential and imperative call for the discovery of new motifs for the infectious treatment with diverse modes of action that possibly will target the resistant and sensitive microbial stains together.[21] When considering the real picture of solving this resistance problem, the screening for potential antimicrobial agents among the novel courses of chemical entities is one of the promising approaches.[22] Considering all the above mentioned issues into account chemists around the world reported various methods for the construction of the indazole heterocycles. Very recently our group reported the synthesis and anticancer activity of *N*-methyl-3-aryl-indazole derivatives using Pd catalyst.[23] In continuation to our efforts in developing structurally diverse heterocyclic compounds as antimicrobial agents[24-27] herein, we report the antimicrobial activities of some *N*-methyl-3-arylindazoles **(5a-5j)**.

EXPERIMENTAL

Recently our group has reported[23] the construction of various *N*-methyl-3aryl-indazole derivatives **5a-5j** using the below Scheme 1 and the structures of the obtained indazoles (Fig. 3), were confirmed by physical parameters like melting point and spectral data.



Scheme 1. Reported pathway for the synthesis of titled indazoles

Reagents and conditions

(i) KOH,I_2, DMF, 25°C, 2 h, 79% (ii) MeI, KOH, acetone, 0°C, Overnight, 60-75% (iii) $Pd(PPh_3)_4$, $NaHCO_3$, DMF, 80°C, Overnight, 60-75%.

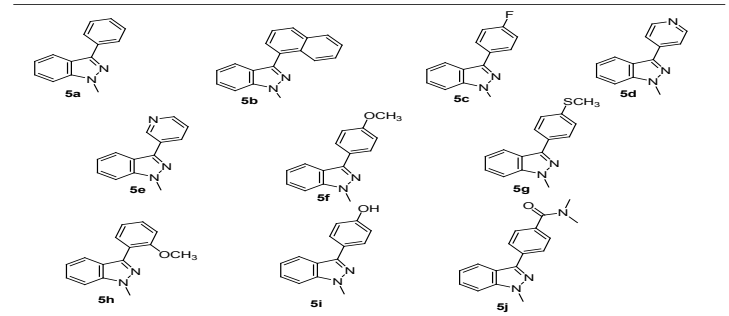


Fig. 3. Prepared indazoles derivatives 5a-5j

MATERIAL AND METHODS

The as synthesized compounds[23] were tested for their antibacterial activity against two types of bacterial strains *Xanthomonas campestris*, *Escherichia coli* (*Gram-negative*) and *Bacillus megaterium* (*Gram-positive*), and also against one fungal strain *Candida albicans* by well diffusion method. The Microbial Type Culture Collection (MTCC) was provided by the Institute of Microbial Technology, based at Chandigarh. All compounds were dissolved in solvent dimethylsulfoxide (DMSO) to get desired final concentration. The antimicrobial activity of the compounds under examination was compared with the activity of the standard antimicrobial drug streptomycin. A control test was also performed with DMSO and found that the solvent did not affect the tested bacteria.

Procedure for antimicrobial activity

The *In vitro* microbial activity of prepared compounds **5a-5j** was determined by the well diffusion method[28]. The antibacterial analysis was done with twenty four hours old bacterial cultures. The pour plate method was used to prepare the required bacterial culture plates for the study. The culture plates were obtained by the addition of about 0.3 mL of each microbial suspension into sterile Petri plates prior to the addition of the molten state nutrient agar. Wells with eight millimeter diameter were prepared by sterile cork borer after solidification of the medium. The samples for investigation were prepared by dissolving 2 mg in 500 µL DMSO. Wells were filled with 100 µL of the sample. At 37°C, each plate was incubated for 24 hours. Following the completion of incubation, the diameter of the zone of inhibition was calculated. Triplicate measurements were done for every sample and microbial species. A similar concentration of standard antibiotic, Streptomycin, was utilized as a positive control. The average zone of inhibition was determined and contrasted with that of the standard. A similar process was followed for examining the antimicrobial activity against the other organisms. Three replicates were maintained for each treatment. Values were given as means ± standard deviation.

To carryout minimum inhibitory concentration analysis, a series of 25, 50, 75, and 100 µL sample dilutions were taken separately in four different test tubes having 10 mL of nutrient broth. A loop full of bacterial inoculum was included in every test tube and then incubated at room temperature for 24 hours. After incubation, the MIC was measured by evaluating the optical density (OD) at 600 nm utilizing a spectrophotometer. The concentration at which minimal growth (OD) of the organism occurred was recorded for each of the tested microorganisms.

RESULTS

Antimicrobial activity

The *In vitro* antimicrobial potency of the synthesized *N*-methyl-3-aryl indazoles **5a-5j** (Fig. 3) was determined by the well diffusion method.[28] The compounds were tested against bacterial strains *Xanthomonas campestris, Bacillus megaterium* (*Gram-positive*), *Escherichia coli* (*Gram-negative*), and the fungal strain *Candida albicans*. Table 1 represents the diameter of zone of inhibition exhibited by the tested compounds for the bacterial strains at a concentration of 100 µL as per the procedure.

Table 1: Antimicrobial evaluation of the test compounds (5a-5j)

S.No.	Compound	Diameter of the Zone of inhibition (in cm) XC[a]	EC[b]	BM[c]	CA[d]
1	5a	2.1± 0.02	1.5± 0.10	1.5± 0.10	1.4± 0.10
2	5b	1.3± 0.01	1.2± 0.10	--	1.6± 0.10
3	5c	1.3± 0.10	1.4± 0.11	1.1± 0.10	1.5± 0.10
4	5d	1.6± 0.11	1.4± 0.11	1.1± 0.11	1.5± 0.11
5	5e	1.7± 0.10	1.1± 0.13	--	1.3± 0.10
6	5f	2.2± 0.11	1.3± 0.14	1.2± 0.10	1.4± 0.10
7	5g	1.5± 0.14	1.4± 0.02	1.1± 0.05	1.3± 0.10
8	5h	1.6± 0.13	1.3± 0.12	1.2± 0.06	1.5± 0.11
9	5i	2.3± 0.14	1.4± 0.20	1.3± 0.09	1.2± 0.11
10	5j	1.4± 0.14	1.6± 0.02	1.6± 0.09	1.5± 0.20
Streptomycin (Standard as positive control)		2.8± 0.15	3.9± 0.02	3.7± 0.12	3.8± 0.12

XC[a]-*Xanthomonas campestris*; EC[b]-*Escherichia coli*; BM[c]-*Bacillus megaterium*; CA[d]-*Candida albicans*
--: no zone of inhibition. *Results are expressed as Mean± Standard deviation of three replicates

The outcome of biological studies of the tested compounds **5a-5j** reveals that they possess antimicrobial activities. From the results of this screening, it was found that the compounds **5i, 5f,** and **5a** showed superior activity against *Xanthomonas campestris* with zone of inhibition as 2.3, 2.2, 2.1 cm respectively, when compared with the zone of standard streptomycin as 2.8 cm. The compounds **5j, 5a,** and **5h** showed excellent activity against *Bacillus megaterium* with zone of inhibitions 1.6, 1.5 and 1.2 cm respectively compared with the zone of standard streptomycin as 3.7 cm. The compounds **5j** and **5a** showed good activity against *Escherichia coli* with zone of inhibitions 1.6 cm and 1.5 cm compared with the zone of standard streptomycin as 3.9 cm. While the compounds **5b**, **5c** and **5d** are moderately active against *Candida albicans* with a zone of inhibition as 1.6 cm, 1.5 cm, and 1.5 cm respectively compared with the zone of standard of streptomycin as 3.8 cm. Based on the zone of inhibition studies it was assumed that (i) the presence of electron donating methoxy and hydroxyl groups at fourth position of the phenyl ring **(5f, 5i)** is accountable for better antimicrobial activity (ii) also an unsubstituted phenyl attached to indazole C_3-position **(5a)** showed good antimicrobial activity.

Minimum Inhibitory Concentration (MIC)

Based on the above results, the authors further tested the compounds **5a-5j** for the minimum inhibitory concentration (MIC) of those compounds for which a high zone of inhibition was recorded in the above test, to control the tested microbes *Xanthomonas campestris, Escherichia coli, Bacillus megaterium* and *Candida albicans*. The MIC values for the tested compounds were presented below in Table 2.

Table 2: MIC test for test compounds 5a-5j along with Zone of inhibition (in cm)

Compound	Concentration of the compound (µL) Organism growth (OD) at different concentrations of compound				Zone of inhibition (diameter in cm)
	25µL	50µL	75µL	100µL	
Bacillus magaterium					
5j	0.213	0.152	0.090	--	1.6
Candida albicans					
5b	0.109	0.108	---	--	1.6
5c	0.426	0.225	0.054	--	1.5
5d	0.105	0.032	--	--	1.5
Xanthomonas campestris					
5a	0.100	--	--	--	2.1
5e	0.956	0.795	0.096	--	1.7
5f	0.332	--	--	--	2.2
5h	0.413	0.263	0.103	--	1.6
5i	0.514	--	--	--	2.3

--: no zone of inhibition

DISCUSSION

The above antimicrobial screening data demonstrated that the MIC of compound **5j** to prevent the growth of *Bacillus megaterium* was 100 µL. Similarly, the growth of *Candida albicans* was prevented at a MIC of 100µL of **5c** and at 75 µL of **5b** and **5d** compounds. However, to control the Xanthomonas campestris the MIC of 50µL of **5a**, **5f**, and **5i** compounds was sufficient. From these studies it can be concluded that (a) samples **5a, 5f, 5i** showed potential antimicrobial activity at low MIC levels to control the gram negative bacterium, *Xanthomonas campestris*; (b) Compounds **5b** and **5d** showed potential antimicrobial activity at low MIC levels to control the pathogenic fungi *Candida albicans*.

CONCLUSION

The synthesis of indazole derivatives inspires us to develop the heterocycles which are having therapeutic importance using simple reagents. Initially, diverse analogs of *N*-methyl-3-aryl indazoles were designed and synthesized. Next, the antimicrobial activities of the obtained analogs were tested. Most of the compounds are active against various bacterial strains. The compounds **5a, 5b, 5i,** and **5j** showed excellent inhibitory activity against different tested microbial strains. Finally, we believe that this call of indazole derivatives presents an interesting profile to promote experimental investigations predominantly in the area of antimicrobial research.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Mills, A.D.; Nazer, M.Z.; Haddadin, M.J.; Kurth.; M. J. *J. Org. Chem.,* **2006**, *71*, 2687-2689.
2. Zhang, T.; Bao, W. *J. Org. Chem.,* **2013**, *78*, 1317-1322.
3. Saczewski, F.; Kornicka, A.; Rybczyn´ska, A.; Hudson, A.L.; Miao, S.S.; Gdaniec, M.; Boblewski, K.; Lehmann, A. *J. Med. Chem.,* **2008**, *51*, 3599-3608.
4. Villanueva, J.P.; Mulia, L.Y.; Sanchez, I. G. *Molecules*., **2017**, *22*, 1864.
5. Kim, S.H.; Markovitz, B.; Trovato, R.; Murphy, B.R.; Austin, H.; Willardsen, A.J.; Baichwal, V.; Morham, S.; Bajji, A. *Bioorg. Med. Chem. Lett*., **2013**, *23*, 2888-2892.
6. Li, X.; Chu, S.; Feher, V.A.; Khalili, M.; Nie, Z.; Margosiak, S.; Nikulin, V.; Levin, J.; Sprankle, K.G.; Tedder, M.E.; Almassy, R.; Appelt, K.; Yager, K.M. *J. Med. Chem.,* **2003**, *46*, 5663-5673.
7. Sun, Y.; Shan, Y.; Li, C.; Si, R.; Pan, X.; Wang, B.; Zhang,J. *Eur. J. Med. Chem.,* **2017**, *141*, 373-385.
8. Chu, Y.Y.; Cheng, H.J.; Tian, Z.H.; Zhao, J.C.; Li, G.; Chu, Y.Y.; Sun, C.J.; Li, W.B. *Chem. Biol. Drug Des*., **2017**, *90*, 609-617.
9. Karalı, N.; Gürsoy, A.; Kandemirli, F.; Shvets, N.; Kaynak, F.B.; Ozbey, S.; Kovalishyn, V.; Dimoglo. A. *Biorg. Med. Chem.,* **2007**, *15*, 5888-5904.
10. Lin, Y.C.; Chou, L.C.; Chen, S.C.; Kuo, S.C.; Huang, L.J.; Gean, P.W. *Bioorg. Med. Chem. Lett*., **2009**, *19*, 3225-3228.
11. Gerpe, A.; Aguirre, G.; Boiani, L.; Cerecetto, H.; González, M.; Azar, C.O.; Rigol, C.; Maya, J.D.; Morello, A.; Piro, O.E.; Aran, V.J.J.; Azqueta, A.; Cerain, A.L.; Monge, A.; Rojas, M. A.; Yaluff, G. *Bioorg. Med. Chem.,* **2006**, *14*, 3467-3480.
12. Angelis, M.D.; Stossi, F.; Carlson, K.A.; Katzenellenbogen, B.S.; Katzenellenbogen, J.A. *J. Med. Chem*., **2005**, *48*, 1132-1144.
13. Andreonati, S.; Sava, V.; Makan, S.; Kolodeev, G. *DiePharmazie*., **1999**, *54*, 99-101.
14. Clutterbuck, L.A.; Posada, C.G.; Visintin, C.; Riddal, D.R.; Lancaster, B.; Gane, P.J.; Garthwaite, J.; Selwood, D.L. *J. Med. Chem.,* **2009**, *52*, 2694-2707.
15. Yakaiah, T.; Lingaiah, B.P.V.; Naraiah, B.; Kumar, K.P.; Murthy, U.S. *Eur J Med Chem.,* **2008**, *43*, 341-347.
16. Minu, M.; Thangadurai, A.; Wakode, S.R.; Agrawal, S.S.; Narasimhan, B. *Bioorg Med Chem Lett.,* **2009**, *19*, 2960-2964.
17. Parekh, C.; Modi, A.; Pillai, J.; Patel, B.A. *Int. J. Drug Res. Tech*., **2012**, *02*, 279-288.
18. Ghaemi, M.; Pordel, M. *Chem. Heterocycl. Comp.,* **2016**, *52*, 52-57.
19. Du, S.J.; Lu, H.Z.; Yang, D.Y.; Li, H.; Gu, X.L.; Wan, C.; Jia, C.Q.; Wang, M.; Li, X.Y.; Qin, Z.H. *Molecules*., **2015**, *20*, 4071-4087.
20. Actelion Pharmaceuticals Ltd., US 9, **2017**, *624*, 206.
21. Holmes, A.; Moore, L.; Sundsfjord, A.; Steinbakk, M.; Regmi, S.; Karkey, A.; Guerin, P.; Piddock, L. *Lancet*., **2016**, *387*, 176-187.
22. Payne, D.J.; Gwynn, M.N.; Holmes, D.J.; Pompliano, D.L. *Nat. Rev. Drug Discov*., **2007**, *6*, 29-40.
23. Rao, S.J.M.; S N Murthy, B.; Raghuram, M.; Adil, S.F.; Rafi, S.M.; Alduhaish, O.; Siddiqui, M.R.H.; HariBabu, B. *Applied Sciences*., **2020**, *10*, 3792.
24. Bhavanam, L.R.; Kotra, V.; Reddy, M.S.; Krishna, K.B.M.; Hari Babu B. *Chemistry Select*., **2019**, *4*, 441-450.
25. Ravi Kumar, G.; Kurmarayuni, C.M.; Manideepa, I.; Ramya Krishna P.; HariBabu B. *Mediterranean Journal of Chemistry*., **2019**, *8*, 261-269.
26. Mutchu, B.R., Kotra, V., Onteddu, S.R., Boddapati, S.N.M., HariBabu B. *Chemistry Africa*., **2019**, *2*, 15-20.
27. HariBabu, B., Narayana,K.J.P., Satyanarayana, P.V.V., *Journal of Pure and Applied Microbiology*., **2009**, *3*, 215-218.
28. Valgas, C.; D'Souza, S.M.; Smania, E.F.; Smania, A. *J. Brazilian Journal of Microbiology*., **2007**, *38*, 369-380.